

Genetic Analysis of the
West Douglas CO feral
Horse Herd

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The following is a report of the genetic analysis of the West Douglas, CO feral horse herd.

METHODS

A total of 32 blood samples were received by the Univ. of Kentucky on October 13, 2001. Seventeen genetic marker systems were analyzed. Seven systems were red blood cell alloantigen loci (the *A*, *C*, *D*, *K*, *P*, *Q* and *U* horse blood groups) tested by standard serological methods of agglutination and complement mediated hemolysis. The other 10 systems were biochemical polymorphisms detected by electrophoretic techniques. These systems were Albumin (*ALB*), Alpha-1-beta Glycoprotein (*AJB*), Serum Cholinesterase (ES), Vitamin D Binding Protein (GC), Glucose Phosphate Isomerase (*GPI*), Alpha Hemoglobin (*FIB*), Phosphoglucomutase (*PGM*), Phosphogluconate Dehydrogenase (*PGD*), Protease Inhibitor (PI), and Transferrin (*TRF*). In addition to the above genetic systems, DNA was extracted from the blood samples and tested for variation at 12 equine microsatellite (mSat) systems. These were *AHT4*, *AHT5*, *ASB2*, *ASB17*, *ASB23*, *HMS3*, *HMS6*, *HMS7*, *HTG4*, *HTG10*, *LEX33*, and *VHL20*. These systems were tested using an automated DNA sequencer to separate Polymerase Chain Reaction (PCR) products.

A variety of genetic variability measures were calculated from the gene marker data. The measures were observed heterozygosity (*Ho*) which is the actual number of loci heterozygous per individual and is based upon biochemical loci only; expected heterozygosity (*He*) which is the predicted number of heterozygous loci based upon gene frequencies and was calculated for biochemical loci and all marker systems (*Het*); effective number of alleles (*Ae*) which is a measure of marker system diversity; total number of variants (*TAW*); estimated inbreeding level (*Fis*) which is calculated as $1 - Ho/He$. These same measures were calculated for the mSat data. However, the DNA data will not be reported due to limited comparative information.

Genetic markers also can provide information about ancestry in some cases. Genetic resemblance to domestic horse breeds was calculated using Rogers' genetic similarity coefficient, S. This resemblance was summarized by use of a restricted maximum likelihood (RML) procedure.

RESULTS AND DISCUSSION

Variants present and allele frequencies for the blood group and biochemical markers are given in Table 1. No variants were observed which have not been seen in horse breeds. Table 2 gives the values for the genetic variability measures of the West Douglas horse herd. Also shown in Table 2 are values for other Colorado feral horse populations plus values from a representative group of domestic horse breeds. The breeds were selected to cover the range of variability measures in domestic horse populations. Mean values for feral herds (based upon data from 54 herds) and mean values for domestic breeds (based upon 118 domestic horse populations) also are shown.

Mean genetic similarity of the West Douglas herd to domestic horse breed types are shown in Table 3. Table 4 shows the genetic similarity matrix for comparison of Colorado feral populations to each other. A dendrogram of relationship of the West Douglas herd to a standard set of domestic breeds (some breeds included in the analysis are not shown individually but are grouped into a breed class for the tree) is shown in Figure 1. This is a consensus tree from 20 individual RML runs. The numbers in the tree are the percentage of runs where the grouping to the right of the number occurred. Figure 2 shows the relationships among the Colorado feral herds.

Genetic variants: Two genetic variants that are uncommon in domestic horse breeds were

observed in the West Douglas herd. These were the *PGD-D* variant and the *A-be* variants, each seen in two individuals. The *PGD-D* variant does not seem to be associated with any particular breed type while the *A-be* variant is extremely rare.

A total of 57 variants were observed which is between the mean values of feral populations and domestic breeds. Of these, 17 occurred at a frequency of less than 0.05 and thus are at high risk of loss. This high proportion of rare alleles suggests a diverse origin of the herd.

Genetic variation: Individual variation of the West Douglas herd is extremely low (H_o 0.269). This is the lowest variation seen in any of the Colorado herds and among the lowest observed in any horse population. H_e also is low but is somewhat higher than H_o indicating some inbreeding.

There is a high degree of allelic diversity however, as indicated above; much of the diversity is due to variants present only at very low frequency. The overall pattern of variability suggest a large population that has been reduced in size and has experienced a loss of genetic variation due to both genetic drift and inbreeding.

Genetic similarity: Highest genetic similarity of the West Douglas herd was with the Gaited North American Breeds followed by the Iberian Breeds. Highest individual breed S was with the Mountain Pleasure Horse which is a breed that shows affinity to much of the North American riding stock. This resemblance is supported by the position of the herd in the dendrogram (Figure 1). The origin of this herd is probably North American riding stock. The Iberian similarity is probably due to the Spanish ancestry of many of the North American breeds rather than direct Spanish ancestry of the West Douglas herd.

The West Douglas herd has highest similarity to the Little Bookcliffs herd among the other Colorado populations. This was followed by the Sand Wash samplings and the 2000 sample from the Spring Creek HMA. These results are somewhat surprising as the other herds with lower S are geographically closer to the West Douglas herd. This discrepancy may be

due to sample sizes and low overall genetic variation. However, examination of the individual types for each of the herds in the White River Resource Area and West Douglas does not reveal any evidence of direct relationship.

SUMMARY

The West Douglas herd has extremely low genetic variation, well below the proposed critical level for H_o of 0.31. Allelic diversity is relatively high but a large proportion of the observed variants are at high risk of loss. The loss of these alleles would likely lead to even lower heterozygosity. The pattern of variation suggest low effective population size and some inbreeding. Genetic similarity values and the RML cluster analysis indicate that this herd is primarily derived from North American riding horse breeds.

RECOMMENDATIONS

Maximum possible population size for this HMA should be maintained after introduction of some horses from outside the West Douglas area. Any horses from the White River Resource Area would be a good choice based upon S values. Three to four young mares would be an effective choice. The herd should be monitored for potential defects or reproductive problems that could arise from inbreeding.

Table 1. Allele frequency for variants observed within the West Douglas, co HMA in 2001.

System/	Allele	Frequency
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Tf	D	.359
	F2	.391
	H2	.047
	O	.156
	R	.047
A1B	K	.969
	S	.031
Es	G	.016
	2	.984
Al	A	.219
	B	.781
Gc	F	.844
	S	.156
PGD	D	.031
	F	.953
	S	.016
PGM	F	.203
	S	.797
GPI	I	1.000
Hb	BI	.875
	BII	.125
Pi	G	.313
	H	.078
	L	.141
	L2	.109
	N	.016
	P	.063
	R	.156
	S	.047
	T	.063
	U	.016
	A	.388
	adf	.032
	adg	.032
	b	.032
	c	.032
	e	.049
	be	.031
C		.436
	a	.646
D		.354
	d	.172
	dk	.141
	dghm	.375
	deo	.078
	bcm	.063
	cgm	.172
K	-	1.000
P	ac	.102
	ad	.102
	b	.047
	-	.749
Q	abc	.016
	b	.232
	c	.016
	-	.736
U		.470
	a	.530

Table 2. Measures of genetic variation of feral horse herds from Colorado and mean values for North American Feral horses and Domestic horse breeds

Herd	<i>N</i>	<i>Ho</i>	<i>He</i>	<i>Het</i>	<i>Fis</i>	<i>TNV</i>	<i>Ae</i>
West Douglas	32	0.269	0.285	0.356	0.058	57	2.202
Sand Wash 2001	50	0.372	0.398	0.425	0.065	63	2.541
Spring Creek Basin	75	0.332	0.331	0.366	-0.004	58	2.038
Barcus Creek WRRRA	37	0.311	0.348	0.364	0.107	56	1.972
Greasewood WRRRA	11	0.345	0.287	0.298	-0.202	41	1.752
Hammond WRRRA	9	0.322	0.286	0.322	-0.127	38	1.817
Little Duck Creek WRRRA	15	0.287	0.327	0.347	0.123	47	1.873
84 Mesa WRRRA	18	0.340	0.383	0.349	0.112	54	2.046
Spring Creek WRRRA	5	0.300	0.248	0.265	-0.210	30	1.664
Square S Well WRRRA	16	0.313	0.304	0.312	-0.025	44	1.917
West Fork Spring Creek WRRRA	15	0.371	0.392	0.364	0.053	43	2.127
Little Bookcliffs	50	0.300	0.299	0.389	-0.004	59	2.322
Sand Wash	72	0.390	0.401	0.450	0.027	71	2.529
Feral Horse Mean	54	0.360	0.351	0.385	-0.035	53.50	2.218
Standard Deviation		0.051	0.053	0.067	0.118	12.50	0.339
Domestic Horse Mean	118	0.371	0.365	0.414	-0.014	65.40	2.358
Standard Deviation		0.049	0.043	0.035	0.065	11.10	0.253

Table 3. Rogers' genetic similarity of the West Douglas feral horse herd to major groups of domestic horses.

	Mean S	Std	Minimum	Maximum
Light Racing and Riding Breeds	0.806	0.023	0.764	0.844
Oriental and Arabian Breeds	0.812	0.033	0.745	0.858
Iberian Breeds	0.818	0.022	0.777	0.864
North American Gaited Breeds	0.828	0.034	0.765	0.868
Heavy Draft Breeds	0.800	0.032	0.720	0.845
True Pony Breeds	0.790	0.035	0.727	0.836

Table 4. Matrix of genetic similarity among Colorado(Feral horse populations.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1.000													
2	.	8351.000												
3	.	844	.8391.000											
4		756	.811	.8291.000										
5	.	787	.800	.781	.8081.000									
6	.	752	.818	.791	.868	.7921.000								
7	.	722	.739	.723	.744	.774	.7211.000							
8	.	754	.711	.765	.676	.666	.690	.6551.000						
9	.	762	.785	.801	.896	.795	.828	.715	.6701.000					
10		776	.792	.821	.907	.798	.835	.716	.710	.9051.000				
11	.	747	.758	.717	.719	.740	.705	.645	.713	.685	.7071.000			
12	.	834	.918	.843	.777	.774	.777	.735	.722	.747	.756	.7381.000		
13		755	.817	.812	.826	.783	.823	.713	.688	.812	.811	.714	.7851.000	
14		.824	.816	.862	.822..795	.777	.731	.734	.787	.796	.722	.813	.8541.000	

1-WEST DOUGLAS

2-SAND WASH 2

3-LITTLE BOOKCLIFFS

4-BARCUS CREEK

5-84 MESA COLORADO

6-SQUARE S WELL AREA

7-WEST FORK

8-HAMMOND AREA

9-GREASEWOOD AREA

10-LITTLE DUCK CREEK

11-SPRING CREEK, WRRRA

12-SAND WASH 1

13-SPRING CREEK 1

14 SPRING CREEK 2000

Figure 1. Dendrogram of Genetic similarity of the West Douglas feral horse herd to domestic horse breeds.

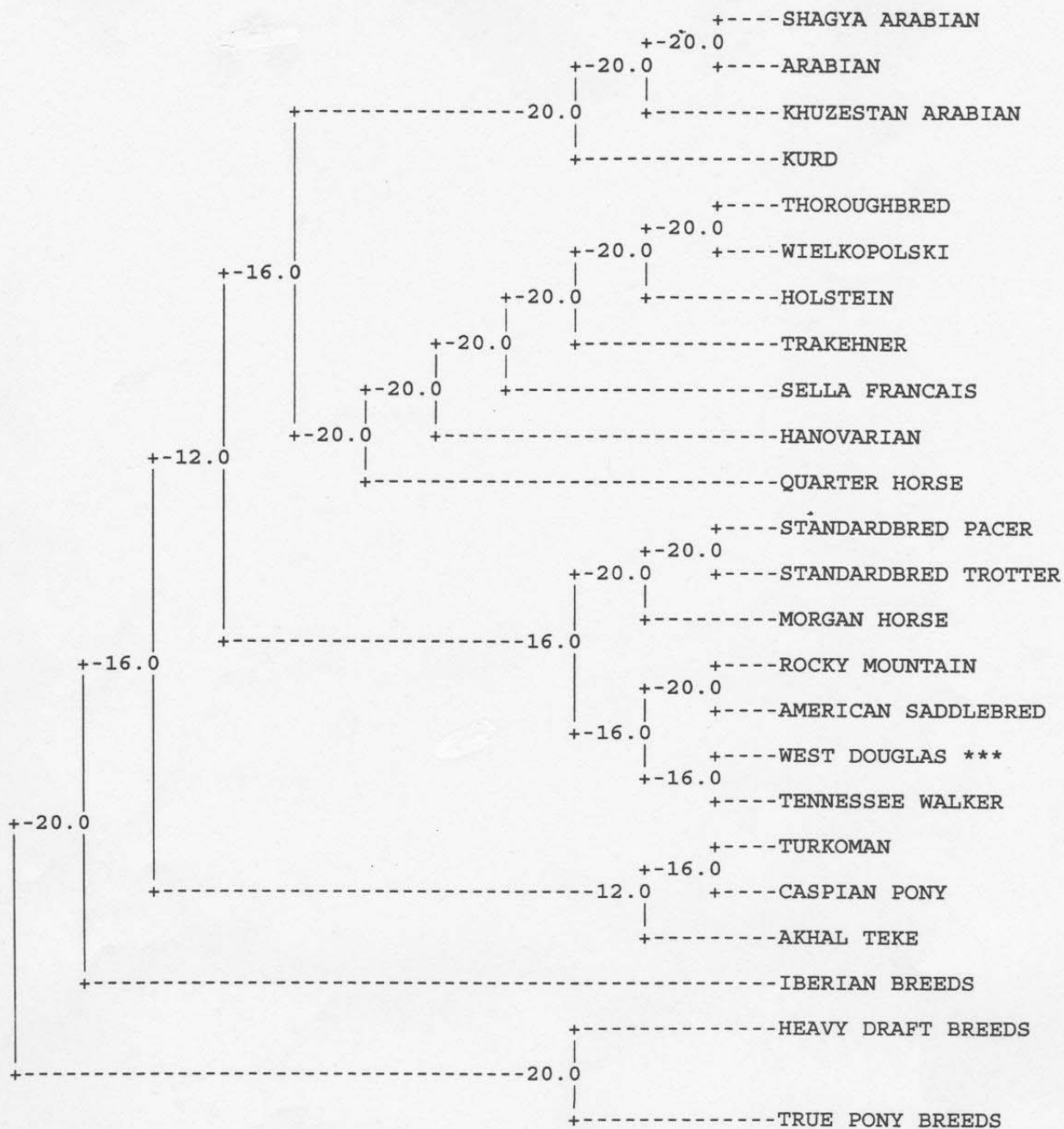
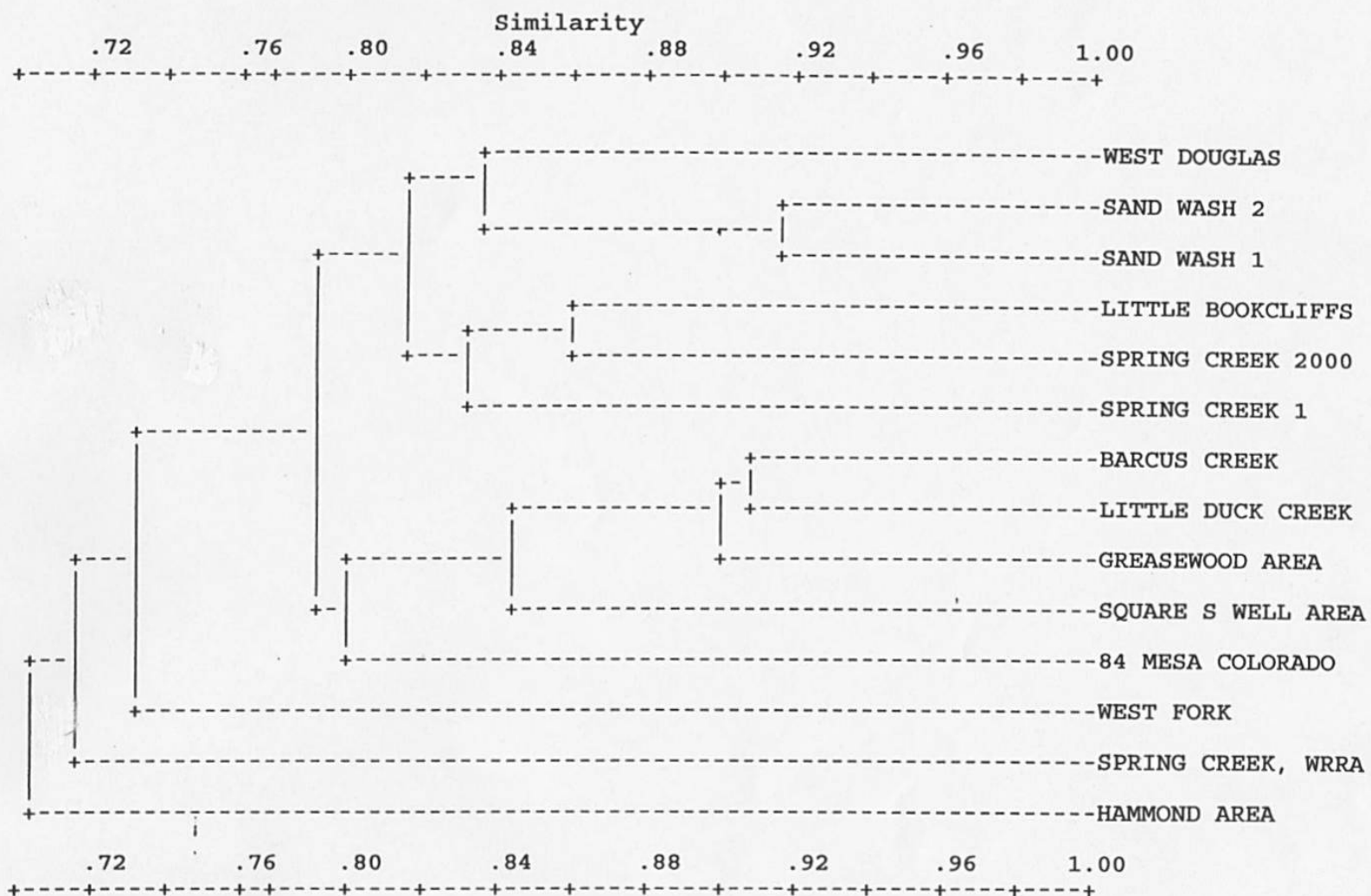


Figure 2. Dendrogram of genetic similarity among Colorado feral horse herds.



Appendix 1. Individual genetic types for West Douglas horses

ID	Biochemical Genetic Systems											Blood Group Systems							
	TRF	A1B	EST	ALB	GC	PGD	PGM	GPI	HB	PI		A	C	D		K	P	Q	U
01-14669	D F2	K K	I I	B B	F F	F F	S S	I I	B1B1	G R		a--d-	- a	--cd--g-	k m--	- -	- -	- -	a
01-14670	F2O	K K	I I	B B	F F	F F	S S	I I	B1B2	G H		a--d-	- a	--cd--g-	k m--	- a-	- -	- -	a
01-14671	D F2	K K	I I	B B	F F	F F	S S	I I	B1B2	H L		a--d-	g a	---de-gh	- m-o	- -	- -	- b-	-
01-14672	D O	K K	I I	A B	F F	F F	S S	I I	B1B1	L2P		a--d-	- a	---d--gh	- m--	- -	- -	- -	a
01-14673	D F2	K K	I I	B B	F F	F F	S S	I I	B1B1	G R		a--d-	- a	---d--gh	- mn-	- -	- -	- b-	-
01-14674	D F2	K K	I I	B B	F F	F F	S S	I I	B1B1	G L		a--d-	- a	---d--gh	k m--	- a-	- b-	- -	
01-14675	D D	K K	I I	A B	F F	F F	S S	I I	B1B1	R S		a--d-	- a	--cd--gh	- m--	- a-	- -	- -	a
01-14676	F2R	K K	I I	A A	S S	F F	F F	I I	B1B1	G G		a--d-	- a	---d--gh	- m--	- -	- -	- -	-
01-14677	D D	K K	G I	A B	F S	F F	S S	I I	B1B1	L2R		-----	- a	--cd--g-	- mn-	- a-	- -	- -	a
01-14678	F2F2	K S	I I	A B	F S	F F	S S	I I	B1B1	L L2		--c--	- a	-bcd--gh	- m--	- -	- -	- b-	a
01-14679	F2H2	K K	I I	B B	F F	F F	S S	I I	B1B1	G G		-----	- a	--cd--g-	k m--	- -	- -	- -	a
01-14680	F2F2	K K	I I	B B	F F	F F	S S	I I	B1B2	G L2		a--d-	- a	---d--gh	k m--	- -	- -	- -	a
01-14681	D F2	K K	I I	B B	F F	F F	S S	I I	B1B1	G G		-----	- a	--cd--g-	- mn-	- -	- -	- -	a
01-14682	D F2	K K	I I	A B	F S	F D	S S	I I	B1B1	T T		-----	- a	--cd--gh	- m--	- -	- -	- b-	-
01-14683	O O	K K	I I	B B	F S	F F	S S	I I	B1B1	G R		a--d-	- a	-bcd--gh	- m--	- a-	- b-	- a	
01-14684	D D	K K	I I	B B	F F	F F	S S	I I	B1B1	G H		a--d-	- a	---d--gh	- mn-	- ab	- b-	- -	
01-14685	F2O	K K	I I	A B	F S	F F	S S	I I	B1B1	H L		a--d-	- -	---d--gh	k m--	- -	- -	- -	a
01-14686	F2F2	K K	I I	B B	F F	F F	S S	I I	B1B1	L P		a--d-	- -	---d--gh	- mn-	- -	- -	- -	a
01-14687	D F2	K K	I I	A A	F F	F S	S S	I I	B1B1	L N		ab-d-	- a	---d----	- -n-	- a-	abc	- -	
01-14688	D F2	K K	I I	A B	F S	F F	S S	I I	B1B1	L2R		-----	- a	---d----	k ---	- -	- -	- -	a
01-14689	F2O	K K	I I	B B	F F	F F	S S	I I	B1B1	G L2		-----e	- -	---de----	- -no	- -	- -	- -	a
01-14690	O O	K K	I I	A B	F S	F F	S S	I I	B1B1	G P		-----e	- a	---de----	- -no	- ab	- b-	- a	
01-14691	H2H2	K K	I I	B B	F F	F F	S S	I I	B2B2	S U		-b---	- a	---de----	- -no	- -	- -	- bc	-
01-14692	D O	K K	I I	B B	F F	F F	S S	I I	B1B1	G S		-----	- -	---de----	k --o	- -	- -	- -	a
01-14693	D F2	K K	I I	A B	F F	F F	S S	I I	B1B2	G L		a--d-	- a	---d--gh	- m--	- a-	- b-	- -	
01-14694	D F2	K K	I I	B B	F F	F F	S S	I I	B1B1	L R		ab-de	- a	--cd--gh	- m--	- ab	- -	- -	a
01-14695	D F2	K K	I I	B B	F F	F F	S S	I I	B1B1	L R		a--de	- a	--cd--gh	- m--	- -	- -	- b-	a
01-14696	R R	K K	I I	B B	F F	F F	F F	I I	B1B1	G R		a--d-	- a	-bcd----	- mn-	- a-	- b-	- a	
01-14697	D F2	K S	I I	A B	F S	F F	S S	I I	B1B1	T T		-bc-e	- a	-bc---g-	- m--	- -	- -	- b-	a
01-14698	D D	K K	I I	B B	F F	F F	S S	I I	B1B2	G R		a--d-	- a	---d--gh	- m--	- a-	- -	- -	a
01-14699	F2F2	K K	I I	B B	F F	F F	S S	I I	B1B2	H L2		a--d-	g a	---d--gh	- m--	- -	- -	- -	a
01-14700	D O	K K	I I	B B	F F	F F	S S	I I	B1B1	G P		a--d-	- a	--cd--gh	- m--	- -	- -	- -	a